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Research Article

Synthesis and Screening of Antibacterial and Antifungal Activity of 6-Amino-4-(Aryl/Heteroaryl)Phenyl-3-Methyl-2,4-Dihydropyrano[2,3-*c*]Pyrazole-5-Carboxamide Derivatives

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Abstract: The present study deals with in vitro antibacterial and antifungal screening of some novel pyrano[2,3-*c*] pyrazole derivatives. A series of novel 6-amino-4-(aryl/heteroaryl) phenyl-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carboxamide derivatives were synthesized by condensation of a mixture of ethyl acetoacetate, aryl/heteroaryl aldehyde, hydrazine hydrate, 2-cyanoacetamide and triethylamine in methanol as a solvent. The structures of all the synthesized compounds are assigned on the basis of IR, ^{1}H NMR and Mass spectral data. Most of the compounds showed mild to moderately active against the *S. aureus* the strain of Gram positive and *E. coli* the strain of Gram negative. The compounds *1d*, *1f* and *1g* exhibited remarkable antifungal activity against *A. niger.*
Keywords: aryl/heteroaryl aldehyde, 2-cyanoacetamide, pyrano [2,3-*c*] pyrazole derivatives, antibacterial and antifungal activity.

INTRODUCTION

Medicinal chemistry is the chemistry discipline concerned with the design, development and synthesis of pharmaceutical drugs [1]. Multicomponent reactions (MCRs) have been known for over 150 years. The first documented multicomponent reaction was the Strecker synthesis of α-amino cyanides in 1850 from which α-amino acids could be derived. MCRs are an important tool in new drug discovery. Multicomponent reactions are those reactions whereby more than two reactants combine in a sequential manner to give highly selective products that retain majority of the atoms of the starting material [2].

Heterocyclic chemistry forms the basis of organic chemistry research worldwide. In particular, heterocyclic structure serves the basis of many pharmaceutical, agrochemical and veterinary products. Among the wide variety of nitrogen heterocycles that have been explored for developing pharmaceutically important molecules, pyranopyrazoles have played an important role in medicinal chemistry [3]. Pyrazoles are the heterocyclic five-membered compounds which possess two nitrogen atoms at 1st and 2nd position of the ring [4]. The term pyrazoles was given by Ludwig Knorr in 1833. Pyrazole and their derivatives exhibit significant biological activities such as antimicrobial [5], anticancer [6], antiviral [7], antidepressant [8] and anticonvulsant [9]. Pyran is an unsaturated heterocyclic compound having a ring containing five carbon atoms and one oxygen atom and two double bonds [10]. Pyrano derivatives have a broad spectrum of biological activities including antimicrobial [12], anticancer [13], antiviral [13] and analgesic [14] activity.

The biological significance of pyranopyrazole derivatives impelled us to continue working on the synthesis of new heterocyclic compounds containing pyrazole and pyran ring system in one molecule resulting in more potent compounds.

MATERIALS AND METHODS

Materials

The chemicals and reagents used in the project work were of AR and LR grade, procured from Astron chemicals, Ahmedabad, Lobachemie private Limited, Mumbai and they are used as they obtained.

Analytical Techniques

Melting points were determined in open capillary tubes and are uncorrected. Compounds were checked for their purity by TLC on silica gel G plates and spots were located by iodine vapors. In some cases TLC GF-254 was used and spots were visualized under UV light.

Instruments

The IR spectra of synthesized compounds were recorded in the range of 4000 – 400 cm^{-1}on FTIR DRS 8400, Shimadzu. Mass spectra were recorded on 2010 EV LCMS Shimadzu instrument at 70eV. ^{1}H NMR

spectra were obtained in $CDCl_3$ on BRUKER Avance-II 400MHz instrument and chemical shift were measured as parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard. Elemental analyses were performed on a Carlo Erba EA 1108 elemental analyser.

METHODS

General procedure for the synthesis of ethyl 6-amino-4-(aryl/heteroaryl)phenyl-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carboxylates (1a-1g)

A mixture of ethyl acetoacetate (0.02 mol, 2.6g, 2.54mL), hydrazine hydrate(99%) (0.02mol, 0.1g, 0.98mL), aryl/heteroaryl aldehyde (0.02mol), ethyl 2-cyanoacetate(0.02 mol, 2.26 g, 2.13mL)and triethylamine (1mL) in methanol (15mL) was refluxed for four hours. The progress of the reaction was monitored by using TLC. The reaction mixture was poured into crushed ice. The solid was obtained and recrystallized.

6-Amino-3-methyl-4-(2,3,4-trimethoxyphenyl)-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carboxamide (1a)

A mixture of ethyl acetoacetate (0.02 mol, 2.6g, 2.54mL), hydrazine hydrate (99%) (0.02mol, 0.1g, 0.98mL), 2,3,4-trimethoxy benzaldehyde (0.02mol, 3.92g), 2-cyanoacetamide(0.02mol, 1.76g) and triethylamine (1mL) in methanol (15mL) was refluxed for four hours. The progress of the reaction was monitored by using TLC. The reaction mixture was poured into crushed ice. The solid was obtained and crystallized using 1,4 - dioxan. Yield: 77%, m.p.: 182-184^{0}C; Rf Value: 0.76; IR (KBr cm^{-1}): 3317, 3294 (NH_2 str.), 3164 (NH str.),1660 (C=O str.), 1602(C=N str.), 1531 (C=C str.), 1362(CH_3 str.),1265, 1165 (C-O-C str.), 1087 (C-N str.);^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:2.0(s, 3H, Ar-CH_3), 3.56(s, 3H, Ar-OCH_3), 3.69(s, 3H, Ar-OCH_3), 3.73(s, 3H, Ar-OCH_3), 4.93(s, 1H, =CH-), 6.64-6.66(d, 1H, Ar-H), 7.12-7.13 (d, 1H, Ar-H); m/z: 360 (M^{+1}); Anal: Calcd for$C_{17}H_{20}N_4O_5$: C, 56.66; H, 5.59; N, 15.55; O, 22.20. Found: C, 56.33; H, 5.23; N, 15.45; O, 22.14.

6-Amino-2,4-dihydro-4-(1*H*-indol-3-yl)-3-methylpyrano[2,3-*c*]pyrazole-5-carboxamide (1b)

A mixture of ethyl acetoacetate (0.02 mol, 2.6g, 2.54mL), hydrazine hydrate (99%) (0.02mol, 0.1g, 0.98mL), indole-3-aldehyde (0.02mol, 2.9g), 2-cyanoacetamide (0.02mol, 1.76g) and triethylamine (1mL) in methanol (15 mL) was refluxed for four hours. The progress of the reaction was monitored by using TLC. The reaction mixture was poured into crushed ice. The solid was obtained and crystallized using rectified spirit. Yield: 70%, m.p.: 174-176^{0}C; Rf Value: 0.55; IR(KBr cm^{-1}): 3421, 3295(NH_2 str.), 3155(NH str.),1666(C=O str.), 1605(C=N str.), 1575 (C=C str.), 1373 (CH_3 str.), 1219,1138 (C-O-C str.), 1093(C-N str.); Anal: Calcd for$C_{16}H_{15}N_5O_2$: C, 62.13; H, 4.89; N, 22.64; O, 10.34. Found: C, 62.09; H, 4.81; N, 22.64; O, 10.34.

6-Amino-4-(2-chloro-6-methylquinolin-3-yl)-2,4-dihydro-3-methylpyrano[2,3-*c*] pyrazole- 5-carboxamide (1c)

A mixture of ethyl acetoacetate (0.02 mol, 2.6g, 2.54mL), hydrazine hydrate (99%) (0.02mol, 0.1g, 0.98mL), 2-chloro-6-methylquinoline-3-carboxaldehyde (0.02mol, 4.00g), 2-cyanoacetamide (0.02mol, 1.76g) and triethylamine (1mL) in methanol (15mL) was refluxed for four hours. The progress of the reaction was monitored by using TLC. The reaction mixture was poured into crushed ice. The solid was obtained and crystallized using rectified spirit. Yield: 87%, m.p.: 187-190^{0}C; Rf Value: 0.74; IR (KBr cm^{-1}): 3402, 3298 (NH_2 str.), 3171(NH str.),1674 (C=O str.), 1597 (C=N str.),1540 (C=C str.), 1381 (CH_3 str.), 1261, 1161 (C-O-C str.), 1068(C-N str.), 739 (C-Cl str.); Anal: Calcd for$C_{18}H_{16}ClN_5O_2$: C, 58.46; H, 4.36; Cl, 9.59 N, 18.94; O, 8.65. Found: C, 58.42; H, 4.31; Cl, 9.54 N, 18.90; O, 8.63.

6-Amino-4-(4-chlorophenyl)-3-methyl-2, 4-dihydropyrano [2,3-c] pyrazole-5-carboxamide (1d)

A mixture of ethyl acetoacetate (0.02 mol, 2.6g, 2.54 mL), hydrazine hydrate (0.02mol, 0.1g, 0.98mL), 4-chlorobenzaldehyde (0.02mol, 2.8g), 2-cyanoacetamide (0.02mol, 2.26 g, 2.13mL) and triethylamine (1mL) in methanol (15mL) was refluxed for four hours. The progress of the reaction was monitored by using TLC. The reaction mixture was poured into crushed ice. The solid was obtained and crystallized using 1,4-dioxan. Yield: 88%, m.p.: 198-200^{0}C; Rf Value: 0.77; IR(KBr cm^{-1}): 3495, 3325 (NH_2 str.), 3115 (NH str.),1671 (C=O str.), 1595(C=N str.), 1396 (CH_3 str.), 1288, 1165 (C-O-C str.), 1087(C-N str.), 734(C-Cl str.); Anal: Calcd for $C_{14}H_{13}ClN_4O_2$: C, 55.18; H, 4.30; Cl, 11.63; N, 18.39; O, 10.50. Found: C, 55.13; H, 4.27; N, 18.35; O, 10.50.

6-Amino-4-(3, 4-dimethoxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carboxamide (1e)

A mixture of ethyl acetoacetate (0.02 mol, 2.6g, 2.54 mL), hydrazine hydrate (99%) (0.02mol, 0.1g, 0.98mL), 3,4-dimethoxy benzaldehyde (0.02mol, 3.32g), 2-cyanoacetamide(0.02mol, 1.76g) and triethylamine (1mL) in methanol (15mL) was refluxed for four hours. The progress of the reaction was monitored by using TLC. The reaction mixture was poured into crushed ice. The solid was obtained and crystallized using rectified spirit. Yield: 87%, m.p.: 187-190^{0}C; Rf Value: 0.74; IR (KBr cm^{-1}): 3411, 3246 (NH_2 str.),3170 (NH str.),1668 (C=O str.), 1598 (C=N str.), 1342 (CH_3 str.), 1234, 1134 (C-O-C str.), 1060(C-N str.); Anal: Calcd for$C_{16}H_{18}N_4O_4$: C, 58.17; H, 5.43; N, 16.96; O, 19.37. Found: C, 58.17; H, 5.49; N, 16.94; O, 19.37.

6-Amino-4-(4-hydroxy-3-methoxyphenyl)-3-methyl-2, 4-dihydropyrano [2,3-*c*]pyrazole-5-carboxamide (1f)

A mixture of ethyl acetoacetate (0.02 mol, 2.6g, 2.54 mL), hydrazine hydrate (99%) (0.02mol, 0.1g, 0.98mL), 4-hydroxy-3-methoxybenzaldehyde (0.02mol, 3.04g), 2-cyanoacetamide(0.02mol, 2.26 g, 2.13mL)and triethylamine (1mL) in methanol (15mL) was refluxed for four hours. The progress of the reaction was monitored by using TLC. The reaction mixture was poured into crushed ice. The solid was obtained and crystallized using rectified spirit. Yield: 74%, m.p.: 205-207^{0}C; Rf Value: 0.71; IR(KBr cm^{-1}): 3415-3175 (OH str.), 3141 (NH str.),1665 (C=O str.), 1619 (C=N str.), 1354 (CH_3 str.), 1219, 1126 (C-O-C str.), 1018(C-N str.); Anal: Calcd for$C_{15}H_{16}N_4O_4$: C, 56.96; H, 5.10; N, 17.71; O, 20.23. Found: C, 56.91; H, 5.07; N, 17.71; O, 20.23.

6-Amino-4-(3-hydroxy-4-methoxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carboxamide (1g)

A mixture of ethyl acetoacetate (0.02 mol, 2.6g, 2.54mL), hydrazine hydrate (99%) (0.02mol, 0.1g, 0.98mL), 3-hydroxy-4-methoxybenzaldehyde (0.02mol, 3.04g), 2-cyanoacetamide(0.02mol, 2.26 g, 2.13mL)and triethylamine (1mL) in methanol (15mL) was refluxed for four hours.The progress of the reaction was monitored by using TLC. The reaction mixture was poured into crushed ice. The solid was obtained and crystallized using rectified spirit. Yield: 76%, m.p.: 203-205^{0}C; Rf Value: 0.70; IR(KBr cm^{-1}): 3421-3188 (OH str.), 3121 (NH str.),1667 (C=O str.), 1621 (C=N str.), 1350 (CH_3 str.), 1265, 1126 (C-O-C str.), 1033(C-N str.); Anal: Calcd for$C_{15}H_{16}N_4O_4$: C, 56.96; H, 5.10; N, 17.71; O, 20.23. Found: C, 56.91; H, 5.08; N, 17.69; O, 20.19.

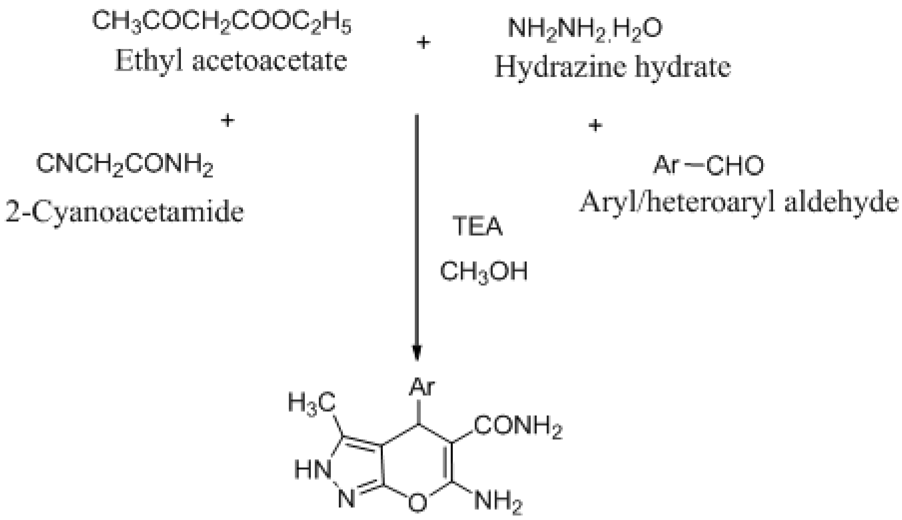
Biological Studies

Antibacterial and antifungal activity by cup plate agar diffusion method

All the newly synthesized compounds have been evaluated for their in vitro for their antimicrobial activity. The antimicrobial activities are carried out against Gram positive bacteria viz., *Staphylococcus aureus* (ATCC 25923) and Gram negative bacteria viz., *Escherichia coli*(ATCC 25922)and antifungal activity towards *Aspergillus niger* (ATCC 16404) at a concentration of 100 µg/mL. The biological activities of synthesized compounds were compared with standard drugs viz., gentamycin, ciprofloxacin, cefotaxime for antibacterial activity and antifungal activity was compared with amphotericine B.

Scheme

Synthesis of 6-amino-4-(aryl/heteroaryl) phenyl-3-methyl-2,4-dihydropyrano[2,3-*c*] pyrazole-5-carboxamides



6-Amino-4-(aryl/heteroaryl)-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole-5-carboxamide

(1a-1g)

a = 2,3,4-trimethoxyphenyl
b = 1*H*-indol-3-yl
c = 2-chloro-6-methylquinolin-3-yl
d = 4-chlorophenyl
e = 3,4-dimethoxyphenyl
f = 4-hydroxy-3-methoxyphenyl
g = 3-hydroxy-4-methoxyphenyl

Table 1: Antimicrobial activity of6-amino-4-(aryl/heteroaryl)phenyl-3-methyl-2,4-dihydropyrano[2,3-*c*] pyrazole-5-carboxamide derivatives at a concentration of 100 µg/mL

Sl. No.	Ar	Zone of Inhibition (mm)		
		S. aureus (Gm +ve)	*E. coli* (Gm -ve)	*A. niger* (Fungus)
1a	2,3,4-trimethoxyphenyl	11	13	14
1b	1*H*-indol-3-yl	09	11	11
1c	2-chloro-6-methylquinolin-3-yl	14	12	15
1d	4-chlorophenyl	10	10	20
1e	3,4-dimethoxyphenyl	10	10	13
1f	4-hydroxy-3-methoxyphenyl	No Zone	No Zone	18
1g	3-hydroxy-4-methoxyphenyl	11	16	18
Gentamycin		20	19	-
Ciprofloxacin		21	28	-
Cefotaxime		33	31	-
Amphotericine B		-	-	20

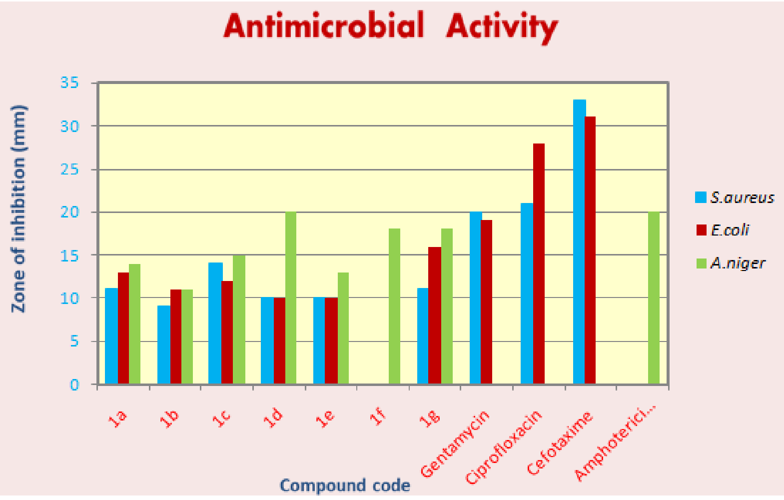


Fig. 1: Antimicrobial activity of synthesized test compounds

DISCUSSION

The novel pyrano [2,3-*c*] pyrazoles (1a-1g) were obtained in good yield by refluxing ethyl acetoacetate, aryl/heteroaryl aldehyde, hydrazine hydrate, 2-cyanoacetamide and triethylamine in methanol as a solvent for four hours. Newly synthesized compounds (1a-1g) were characterized by IR, NMR, mass spectral and C, H, N elemental analyses.

In pyrano [2,3-c] pyrazole dervatives, the compounds 1e and 1f exhibited characteristic band in the frequency region of 3100-3400 cm-^{1} due to 'OH'. The compounds 1a-1d and 1g exhibited characteristic band in the frequency region of 3200-3400 cm-^{1} due to 'NH'. The compounds (4a-4e) showed characteristic band in the frequency region of 1660-1680 cm^{-1} of amide linkage. The compounds 1c and 1g exhibited characteristic band in the frequency region of 700-750cm-^{1} due to 'Cl'. The MASS spectra of compounds were taken in both positive and negative mode. The compound 1a showed molecular ion peak at 360 m/e. The ^{1}H NMR spectra of compound 1a was studied $CDCl_3$. Aryl protons of all compounds resonate at around 6.5-7.2 ppm as multiplet, the proton of methoxy substituent attached to aryl rings were found to appear as singlet at 3.56, 3.69 and 3.73 ppm and the proton of methyl substituent attached to aryl rings was found to appear as singlet at 2.0 ppm. The signal appearing at 4.93 ppm is attributed to methine proton.

The preliminary screening results indicated that the most synthesized compounds showed

antimicrobial activity from mild to moderate. The compound 1aand 1c showed a moderate inhibition against *E. coli* and *A. niger*. The compound 1b, 1d and 1e showed a mild inhibition against *S. aureus* and *E. coli*. The test compound 1d exhibited an equipotent antifungal activity against *A. niger*at100 µg/mL due to the presence of 4-chlorophenyl substituent which suggests that it has good antifungal activity with respect to amphotericine B. However, the compound 1g showed no zone inhibition against *S. aureus* and *E.coli*. But, it showed effective inhibition against *A. niger* due to the 4-hydroxy-3-methyl phenyl substituent which suggests it has more comprehensive fungicidal activity than bacteria inhibitory properties due to the presence of 3-hydroxy-4-methyl phenyl substituent. The compound **1f** showed good inhibition against *A. niger* with respect to amphotericine B which suggests that it has good antifungal activity.

All the compounds were screened for their antibacterial activity by Cup-Plate Method using different strain like *E. coli and S. aureus* at 100 µg/mL. Gentamycin, ciprofloxacin and cefotaxime were taken as the standard drugs for antibacterial activity. Amphotericine B was taken as the standard drugs for antifungal activity. The test compounds 4e (R= 4-hydroxy-3- methoxy phenyl), 4f (R= 3-hdroxy -4-methoxy phenyl) and 4g (R= p-chloro phenyl), the antifungal activity is comparable with amphotericin B against *A. niger* at a concentration of 100 mcg/ml.

CONCLUSION

The novelpyrano [2,3-c] pyrazole derivatives were synthesized and screened for antibacterial and antifungal activity. Among all synthesized compound, most of the compounds showed mild to moderately active against the *S. aureus* the strain of Gram positive and *E. coli* the strain of Gram negative. The compounds *1d*, *1f* and *1g* exhibited remarkable antifungal activity against *A.niger.*

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